

AMENDMENTS TO THE CLAIMS

The listing of claims will replace all prior listings of claims in the application.

Listing of claims:

Claims 1-15 (canceled).

Claim 16 (currently amended): A recombinant nucleic acid molecule encoding a modified ~~type-14~~ pneumolysin polypeptide comprising one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity.

Claim 17 (currently amended): A ~~The recombinant nucleic acid molecule according to claim 16~~ comprising the pneumolysin nucleic acid sequence of SEQ ID NO: 1 or non-coding sequence changes thereof,

and wherein said nucleic acid sequence comprises one or more of the nucleotide substitutions selected from the group consisting of:

A-50→G, G-54→T, T-181→C, A-196→T and T-302→C;

A-122→G, A-514→G, T-583→A and A-764→G;

A-187→T, T-380→A, A-382→C and T-443→A;

T-98→C, T-137→C, T-248→C, T-717→A and A-770→G;

T-134→C, A-305→G, A-566→G and T-583→G;

T-583→G;

T-583→A;

T-443→A;

and T-181→C.

Claim 18 (currently amended): The ~~recombinant-nucleic acid~~ molecule of claim 16 or claim 17 as contained in a vector.

Claim 19 (currently amended): A microorganism comprising the ~~nucleic acid~~ molecule of any of claims claim 16-17 or claims 32-40.

Claim 20 (previously presented): The microorganism according to claim 19, wherein the microorganism is selected from the group consisting of: bacteria, yeast, mammalian and insect cells.

Claim 21 (previously presented): The microorganism according to claim 20, wherein the microorganism is *E. coli*.

Claims 22-26 (canceled).

Claim 27 (previously presented): A method for killing bacteria comprising contacting said bacteria with antibodies to an immunogenic molecule comprising a modified pneumolysin comprising one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity in the presence of

complement.

Claim 28 (original): The method according to claim 27, wherein the immunogenic molecule is a polysaccharide-polypeptide conjugate wherein the polysaccharide is a bacterial capsular polysaccharide.

Claim 29 (previously presented): A method for immunization of mammals comprising administering a vaccine comprising the modified pneumolysin polypeptide comprising one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity and a pharmaceutically acceptable carrier to said mammals.

Claim 30 (previously presented): A method for obtaining modified pneumolysin polypeptides, wherein said modified pneumolysin polypeptides have reduced hemolytic activity and are suitable for eliciting an immunogenetic response which is cross-reactive with wild-type pneumolysin comprising the steps of:

(a) mutating a nucleic acid molecule encoding wild-type pneumolysin to produce mutated nucleic acid molecules encoding modified pneumolysin polypeptides, wherein the modified pneumolysin polypeptides comprise one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position

61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257 and expressing the mutated nucleic acid molecules in host cells;

(b) assaying the modified polypeptide expressed by the host cells for hemolytic activity; and

(c) identifying the modified pneumolysin polypeptides having substantially similar molecular weight as native wild-type pneumolysin and which are refoldable.

Claim 31 (currently amended): ~~The recombinant nucleic acid~~ molecule of claim 16 or claim 17, wherein the vector is selected from the group consisting of: a plasmid, cosmid, bacteriophage and yeast artificial chromosome.

Claim 32 (new): A molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNVJ1.

Claim 33 (new): A molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNVJ20.

Claim 34 (new): A molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNVJ22.

Claim 35 (new): A molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNVJ45.

Claim 36 (new): A molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNVJ56.

Claim 37 (new): A molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNV103.

Claim 38 (new): A molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNV207.

Claim 39 (new): A molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNV111.

Claim 40 (new): A molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNV211.